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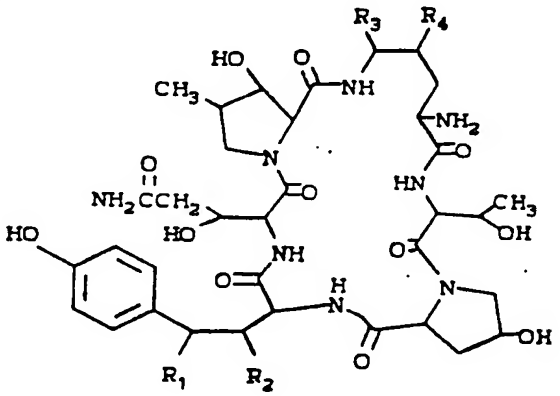
(54) Cyclohexapeptide compounds

(57) Cyclohexapeptides of the formula



wherein (1) R_2 is hydrogen and R_1, R_3 and R_4 are hydroxy. (2) R_1, R_2 and R_4 are hydrogen and R_3 is hydroxy; or (3) R_1, R_2, R_3 and R_4 are hydrogen; and acid addition salts thereof, are useful as intermediates for producing antifungal compounds.

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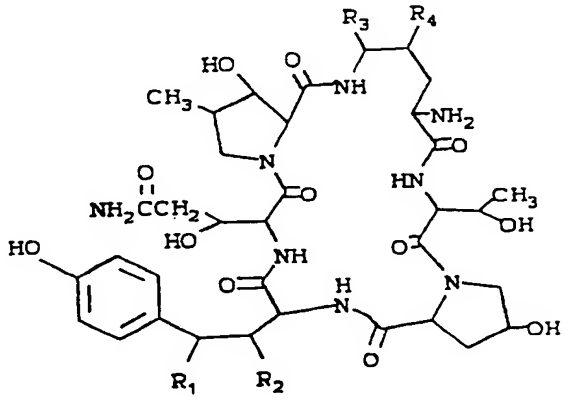
TITLE OF THE INVENTION

CYCLOHEXAPEPTIDE COMPOUNDS

The present invention relates to closely related cyclohexapeptide bases which may be represented by the formula



wherein R_1, R_2, R_3 and R_4 independently are hydrogen or hydroxy, and are selected from one of the following compounds:

(1) R_2 is hydrogen and R_1, R_3 and R_4 are hydroxy, wherein the compound is designated compound IA and may be named 1-(4,5-dihydroxyornithine)-4-[4-hydroxy-4-(4-hydroxyphenyl)-2-aminobutanoic acid]-5-(3-hydroxyglutamine)echinocandin B.

(2) R_1, R_2 and R_4 are hydrogen and R_3 is hydroxy, wherein the compound is designated IB and may be named 1-(5-hydroxyornithine)-4-[4-hydroxy-4-(4-hydroxyphenyl)-2-aminobutanoic acid]-5-(3-hydroxyglutamine)echinocandin B.

(3) R_1, R_2, R_3 and R_4 are hydrogen, wherein the compound is designated IC and may be named 1-(ornithine)-4-[4-(4-hydroxyphenyl)-2-(aminobutanoic acid]-5-(3-hydroxyglutamine) echinocandin B; and acid addition salts thereof.

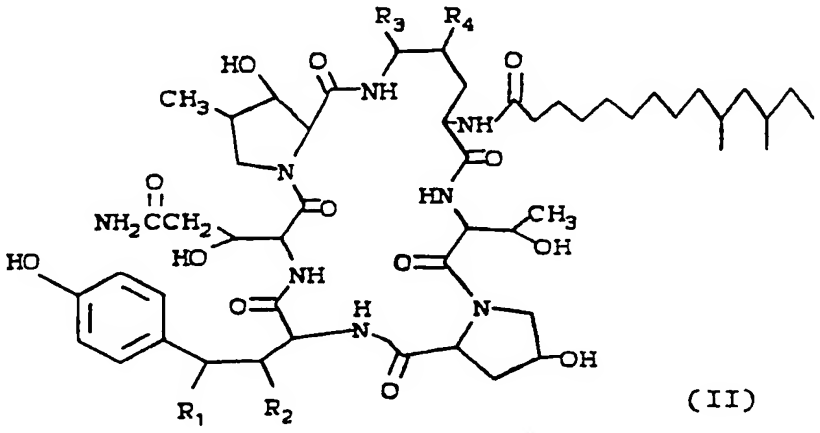
When the expression "Compound I" is hereinafter employed, without the letters A, B, or C it is meant to embrace all three compounds represented by formula (I).

(In the formulas, a hydrogen attached to a carbon is not specifically indicated unless it is one of R_1, R_2, R_3 or R_4).

Compound I may be retained in the form of acid addition salts. Representative acids for forming salts include hydrochloric, hydrobromic hydroiodic, sulfuric, phosphoric, acetic, benzoic,

sulfamic, tartaric, citric, maleic, succinic, ascorbic, glycolic, lactic, fumaric, palmitic, cholic, pamoic, mucic, D-glutamic, D-camphoric, glutaric, phthalic, lauric, stearic, salicylic, methanesulfonic, benzenesulfonic, sorbic, picric, cinnamic, and the like. Compound I and salts thereof are useful as intermediates in the preparation of semisynthetic compounds which have antifungal properties.

The cyclohexapeptide base, Compound I, may be obtained by deacylating a compound having the formula (II)



(II)

and hereinafter referred to as Compound II.

When the expression "Compound II" is employed without the letters A, B, or C, it is meant to embrace all the compounds represented by formula (II). The designation A, B, or C after II designates a Compound II which is a precursor of and which corresponds to the particular deacylated compound in

the nuclear substitution. Compound II is described and claimed in copending application Serial No. 374,418, and copending application Serial No. 495,019, corresponding to EP-A-0405998, and the teachings therein of preparation and properties are incorporated by reference.

Compound I may be prepared by the deacylation of Compound II by subjecting Compound II in an aqueous medium, i.e. a nutrient medium in a buffer solution, to a deacylating enzyme obtained from or present in intact cells of microorganisms of the family Pseudomondaceae or Actinoplanaceae, or a microorganism made to produce the deacylating enzyme through recombinant DNA technology. The deacylation may be monitored by Candida albicans assay or HPLC (high performance liquid chromatography) assay and the conversion allowed to continue until deacylation is complete as indicated by the disappearance of anti-Candidal activity of the substrate (Compound II) or by the appearance of the product (Compound I).

The separation of a mixture of Compound IA, IB and IC is extremely difficult. Thus, if a mixture of Compound II is deacylated, i.e., a mixture of IIA, IIB and IIC, it should be intended that the mixture of Compound IA, IB and IC is to be employed. If one of Compound IA, IB or IC is desired, the starting material preferably is Compound II having the appropriate nucleus since a mixture of Compound IIA, IIB and IIC may be separated by chromatography, for example on a Zorbax (DuPont) ODS column using a solvent mixture of 50:50 acetonitrile/water as fully described in the aforementioned copending application.

Compound I or component then may be isolated from the resulting fermentation broth by centrifuging the broth, recovering the supernatant and passing it through a column of "Diaion" HP-20 or SP-207 (styrene-divinylbenzene copolymer and brominated styrene divinylbenzene copolymer high porous polymer, respectively, Mitsubishi Chemical Industries, Ltd.) to retain Compound I on the column, then after first washing the column with deionized water, eluting with methanol to recover Compound I in the eluate. The eluate fractions are combined and concentrated to obtain crude Compound I. The latter may be purified by cation exchange HPLC followed by desalting on a HP-20 column as hereinafter more fully detailed.

The eluates when acylated with an activated ester of an appropriate fatty acid are converted into compounds which are active against C. albicans and other yeasts as well as against filamentous fungi. Thus, the products of the present invention are useful as intermediates in the preparation of antifungal agents. The preparation and properties of these agents are the subject of copending, concurrently filed application, Serial No. 494,633, (Attorney Docket No. 18035).

PREPARATION OF THE STARTING MATERIAL

The starting material for the deacylation, Compound II, may be obtained by cultivating Zalerion arboricola, ATCC 20868, in a nutrient medium until Compound II is produced, thereafter extracting either the mycelium or whole broth with methanol, vaporizing the solvent to obtain a residue, dissolving the

residue in a solvent suitable for chromatographic separation, and chromatographing to recover Compound II in the eluate.

Z. arboricola ATCC 20868 is described in copending application Serial Nos. 105,795 and 105,797 corresponding to EP-A-0311193 and EP-A-0311194 respectively, and is available from the American Type Culture Collection at 12301 Parklawn Drive, Rockville, MD 20852. The teachings of these applications are incorporated by reference.

The nutrient medium useful for producing Compound II is that supplying carbon, nitrogen and inorganics salts. Sources of carbon may be glycerol, sugars, sugar alcohols, starches, carbohydrate derivatives or may be in complex nutrients such as oat flour, corn meal, millet and the like; sources of nitrogen may be ammonium salts, amino acids such as glycine, threonine, methionine and the like or may be in complex sources such as yeast hydrolysates, yeast extracts, corn steep liquors, cottonseed meal and the like; and inorganic nutrients are supplied in customary salts such as potassium, magnesium, calcium, phosphate, chloride, carbonate, and trace metals. Particularly important salts are ammonium and monobasic potassium phosphate salts.

In carrying out the production, the fermentation medium is inoculated with a culture growth which has been prepared from frozen vegetative mycelia of Z. arboricola in a conventional manner and the fermentation production medium incubated for from 3 to 30 days, with or without agitation at temperatures in the range of from about 20°C to about 40°C at a pH in the range of from about 5.0 to 8.5.

At the end of the cultivation period, the active component is recovered by adding alkanol to the medium if the fermentation had been carried out in a solid medium or to the whole broth if the fermentation had been carried out in a liquid medium. The aqueous alkanol solution is filtered to remove solid impurities and then adsorbed on "Diaion" HP-20 or equivalent styrene-divinylbenzene copolymer and then eluted with 100% alkanol. This may be repeated and the crude isolate mixture subjected to chromatographic separation using conventional column chromatography with non-ionic resin such as silica gel or by high performance liquid chromatography employing reverse phase resin, or a combination thereof. With silica gel, ester/alcohol mixtures provide good separations; with dextran adsorbent a chlorohydrocarbon/hydrocarbon/alcohol system is useful. Fractions containing antibiotic Compound II may be detected by antifungal activity against C. albicans or by analytical HPLC with retention compared to a previously determined standard. The active fractions are combined and concentrated to obtain crude Compound II. The latter may be purified employing conventional techniques such as further chromatography. Also it may be desirable to sterilize Compound II prior to enzymatic deacylation but is not generally required. Since Compound II is the substrate in the deacylation step, in the discussion of the step, Compound II may be referred to as the substrate.

The details of the production and isolation of Compound II are found described in the aforementioned copending application Serial No.

374,418 and copending application Serial No. 495,019 corresponding to EP-A-0405998, and the teachings therein are incorporated by reference.

DEACYLATION

A. Deacylation Enzyme

The enzyme which is useful for deacylation is produced by certain micro-organisms of the family Pseudomondaceae and Actinoplanaceae. The organisms of the Pseudomondaceae family are preferred, especially Pseudomonas acidovorans and Pseudomonas diminuta.

The Actinoplanaceae may be the same enzyme used to deacylate penicillins and described in U.S. patent 3,150,059 or that described in U.S. patent 4,299,763. Among the species and varieties of Actinoplanaceae which may be employed are Actinoplanes philippinensis, Actinoplanes armeniacus, Actinoplanes utahensis, and Actinoplanes missouriensis, Spirillospora albida; Streptosporiangium roseum, Streptosporangium vulgare, Streplosporangium roseum var. hollandensis, Streptosporangium album; Streptosporangium viridialbum, Amorphosporangium auranticolor, Ampullariella regularis, Ampullariella campanulata, Ampullariella lobata, Ampullariella digitata; Pilimelia terevasa, Pilimelia anulata; Planomonospora parontospora, Planomonospora venezuelensis; Planobispora longispora; Planobispora rosea; Dactylosporangium aurantiacum, and Dactylosporangium thailendense.

Cultures of useful species of Pseudomonadaceae or Actinoplanaceae may be obtained from the American Type Culture Collection, address supra. Representative of a preferred culture for the production of the enzyme is P. acidovorans originally obtained as ATCC 11299B from the American Type Culture Collection and maintained as MB 3744 in the culture collection of Merck & Co., Rahway, N.J. A sample of MB 3744 has been resubmitted to the American Type Culture Collection for deposit under the Budapest Treaty and has been assigned accession no. ATCC 53942.

The morphological and cultural characteristics of ATCC 53942 are are as follows:

Gram-negative aerobic rod, approximately 0.8 - 1.0 μm x 3.0 - 4.0 μm. Growth occurs on trypticase soy agar at 25-37°C. Colonies are opaque and convex with an entire margin and glistening surface. Colonies have a butyrous texture. No pigments observed. Growth on MacConkey agar also observed.

The biochemical characteristics of this strain are as follows: oxidase positive, gelatin is hydrolyzed, nitrate reduced to nitrite. Growth occurs by assimilation of the following carbon sources in the presence of ammonium sulfate: D-gluconate, caprate, adipate, and malate D-mannitol, and phenylacetate.

As will be apparent to those in the field, the microorganisms which produce the enzyme are subject to variation. For example, artifical variants and mutants of these strains may be obtained by treatment with various known mutagens such as

ultraviolet rays, X-rays, high-frequency waves, radioactive rays, and chemicals. All natural and artifical variants and mutants of the Pseudomondacea the Actinoplanaceae which produce the enzyme may be used in this invention.

The enzyme may be produced under conditions satisfactory for the growth of the producing organism. For the organism Actinoplanacea, the conditions generally are a temperature in the range 25° to 30°C, and a pH of between about 5.0 and 8.0, employing agitation and aeration. The culture medium should contain (a) an assimilable carbon source such as sucrose, glucose, glycerol, or the like; (b) a nitrogen source such as peptone, urea, ammonium sulfate, or the like; (c) a phosphate source such as soluble phosphate salt; and (d) inorganic salts found generally to be effective in promoting the growth of microorganisms. An effective amount of the enzyme is generally obtained in from about 40 to about 60 hours after the beginning of the growth cycle and persists for some time after the effective growth has been reached.

For the organism Pseudomonas, the conditions generally are a temperature in the range 20° to 40°C, a pH between 5.5 and 8.5 while employing agitation and aeration. The culture medium should contain (a) an assimilable carbon source such as carbohydrates, sugar alcohols and sugar derivatives, fatty acids, dicarboxylic acids, hydroxy acids, aliphatic amino acids, amino acids and related compounds, or the like; (b) a nitrogen source such as beef extract, peptone, yeast extract, soybean digest, casein digest, brain heart infusion, or the like and (c)

inorganic salts found generally to be effective in promoting the growth of micoorganisms. An effective amount of enzyme is generally obtained in from about 16 to about 48 hours after the beginning of the growth cycle.

Representative of a medium suitable for production of a deacylase by the Pseudomonas species is Luria-Bertani medium having the following composition:

	per liter
Bacto-Tryptone	10 g
Bacto-Yeast Extract	5 g
Sodium chloride	10 g

No pH adjustment

Generally, the enzyme is envelope-bound and is not cryptic in the intact cells, thereby permitting use of resting suspensions of washed live cells for deacylation. The amount of enzyme produced varies from species to species of the organism and in response to different growth conditions.

However, instead of growing or resting cells, soluble or immobilized enzymes obtainable by methods known to the skilled artisan may be used. Furthermore, deacylating enzyme produced by recombinant technology with genes obtained from microorganisms also may be employed.

B. Deacylation and Recovery of Compound I

The substrate used as the starting material is preferably added as a solution in dimethylsulfoxide (DMSO) to a resting suspension of washed Pseudomonas acidovorans cells in phosphate buffer pH 6.5, after the cells have been grown in a nutrient medium for 16 to 24 hours. The concentration of substrate in the conversion medium may vary widely. For maximum use of enzyme source and for substantially complete deacylation within a 24-hour period however, the concentration of substrate will generally range from about 0.5 to about 2.0 mg/ml. Lower concentrations may be used but such may not make maximal use of the enzyme; higher concentrations may also be used but the substrate then may not completely deacylate because of its insolubility.

In an alternate procedure, the substrate may be added to a culture of Actinoplanaceae under similar conditions.

The most suitable conditions, not only for converting the substrate antibiotic to a cyclopeptide Compound I but also for the stability of Compound I produced, are when the pH of the reaction medium is maintained in the range of from about 6.0 to about 7.0. A pH of about 6.5 is preferred.

After addition of the substrate, incubation of the culture should be continued for about 24 hours or longer. The purity of the substrate will affect the rate of deacylation. When substrates of lower purity are used, the deacylation proceeds at a slower rate. Multiple additions of substrate may be employed.

The deacylation may be carried out over a broad temperature range, i.e., from about 20° to about 60°C. Preferred temperatures are between 30° and 60°C.

The deacylation may be monitored using a C. albicans assay since Compound II is very active against C. albicans while Compound I is biologically inactive. Both broth and alcoholic extracts of fermentation solids should be assayed since the solid is only slightly soluble in aqueous solutions.

Compound I may be separated from the fermentation broth by centrifuging to separate the cells, adsorbing Compound I onto a chromatographic column, preferably "Diaion" SP-207 or HP-20, and recovering from the resin by eluting with methanol and concentrating the active eluates. Compound I may be further purified by cation exchange preparative HPLC followed by desalting on SP-207.

The following examples illustrate the invention but are not to be construed as limiting.

EXAMPLE I

COMPOUND I
MIXTURE OF
(IA, IB, & IC)

A. Preparation of the Deacylating Enzyme

P. acidovorans ATCC 53942, maintained on slants of Luria-Bertani medium solidified with 2% agar, was employed to produce the deacylation enzyme.

A seed culture was first prepared by inoculating a 50-ml portion Luria-Bertani medium with

a loopful of the bacteria and the culture incubated for 24 hours at 28°C with shaking. Cells for the deacylation were then grown by diluting the seed culture 1:500 into twenty 50-ml portions of fresh Luria-Bertani medium in 250 ml flasks and incubating for 16 hours at 28°C with shaking.

Cells from 1 liter of culture were harvested by centrifugation at 6600 g for 20 minutes. The cells were resuspended in 1% NaCl and again collected by centrifugation at 6600 g for 20 minutes. The cells were then suspended in 475 milliliters of 50 mM potassium phosphate buffer, pH 6.5 and the suspension warmed to 37°C to obtain the deacylating enzyme.

B. Preparation of Compound II (Substrate)

250 ml flasks are prepared containing 54 milliliters of KF seed medium of the following composition:

KF Seed Medium

	per liter	
Corn steep liquor	5.0	g
Tomato paste	40.0	g
Oat flour	10.0	g
Glucose	10.0	g
Trace elements	10.0	ml
Distilled water	1000	ml

pH 6.8

Trace elements	per liter 0.6N HCl	
$FeSO_4 \cdot 7H_2O$	1.0	g
$MnSO_4 \cdot 4H_2O$	1.0	g
$CuCl_2 \cdot 2H_2O$	0.025	g
$CaCl_2$	0.1	g
H_3BO_3	0.056	g
$(NH_4)_6Mo_4O_{27}4H_2O$	0.019	g
$ZnSO_4 \cdot 7H_2O$	0.2	g

The flasks are inoculated from a frozen vegetative mycelium of Zalerion arboricola ATCC 20868 and are then incubated at 25°C for three days at 220 rpm.

A 20 ml sample of the culture is then inoculated into each of four resulting 2 liter flasks containing 500 ml of KF seed medium and cultivated at 25°C for three days while shaking at 220 rpm. The contents are pooled and then inoculated into 180 liters of KF medium containing 2 ml/liter of polypropylene glycol P-2000 (Dow Chemical Co.) to reduce foaming and cultivated for three days at 25°C while agitating at 220 rpm, an air flow of 90 liters/minute and pressure of 0.7 kg/cm^2 gauge. A 25-liter sample of the culture is then inoculated into each of two 475 liters of a TG103 production medium of the following composition (also modified with 2 ml/liter polypropylene glycol P-2000):

TG103 production medium

	per liter	
D-mannitol	40	g
NZ-Amine type E	33	g
Fidco Yeast Extract	10	g
$(NH_4)_2SO_4$	5	g
KH_2PO_4	9	g

No initial pH adjustment;
sterilized 120 C for 25 minutes

The foregoing medium is disclosed in copending applications Serial Nos. 374,418, and 374,416, corresponding to EP-A-0405998 and EP-A-0405997 respectively.

Cultivation is carried out for 5 days at 25°C with agitation at 150 rpm, an air flow of 250 liters per minute air flow, a pressure of 0.7 kg/cm^2 gauge. The pH is allowed to decrease from an initial value of about 5.8 to 5.5, and then maintained at 5.5 ± 0.2 using NaOH and H_2SO_4. After five days the broth is harvested for product isolation.

An equal volume of methanol (MeOH) is added to the fermentation whole broth of two of the 475 liter batches described above and the mixture agitated for 8 hours. This whole broth extract is centrifuged to remove the insoluble fermentation solids and to obtain a clarified supernatant, the pH of which then is adjusted to 7.

A "Diaion" SP-207 bed, corresponding to 1.5 gram of total solids per liter of resin, is prepared by washing with MeOH and pre-equilibrating with 50:50 MeOH/H_2O. The clarified supernatant is then charged to the SP-207 column in an upflow direction at a fluidized bed rate consistent with a 10 to 15 minute contact. After charging, the column is washed with

65:35 MeOH/H_2O and eluted with 100% MeOH. The eluates are concentrated to obtain Compound II (a mixture of IIA, IIB and IIC).

C. Deacylation and Preparation of Compound I

A one gram quantity of Compound II is dissolved in 25 ml of dimethyl sulfoxide and the resulting solution added dropwise to a stirred 475 milliliter suspension of P. acidovorans ATCC 53942 cells containing the deacylating enzyme and the reaction mixture maintained at 37°C for 18 hours whereupon deacylation is found to be complete with the formation of Compound I as determined by a Candida albicans assay.

The reaction mixture is then centrifuged at 6600 G for 20 minutes to separate the cells and to recover Compound I in the supernatant.

The supernatant is adsorbed on HP-20 resin and crude Compound I is recovered by elution with methanol and concentrating the eluates. The eluates are combined, diluted with water, charged to a preparative HPLC system equipped with a 50 cm. Whatman Partisil 20 SCX (strong cation exchange, phenyl-SO_3^-) Magnum 20 column, and then eluted at 20 ml/min with 0.01 M potassium phosphate (pH=6) buffer and monitored via UV at 210 nm. Cuts rich in the deacylated products as indicated in analytical HPLC were combined and the resulting mixture adsorbed and eluted from HP-20 resin with methanol to remove buffer and to obtain Compound I (a mixture of IA, IB and IC).

EXAMPLE II

The contents of one frozen vial of Zalerion arboricola ATCC 20868 are defrosted and aseptically transferred to a 250 milliliter unbaffled flask containing 54 milliliters of KF medium containing 0.4 per cent agar. The modified medium after inoculation is incubated at 28°C with 220 rpm agitation for 48 hours. At the end of this period, 10 milliliters of the growth is transferred to a 2-liter unbaffled flask containing 500 milliliters of KF medium containing 0.4 per cent agar. After inoculation, the resulting medium is incubated for 24 hours at 28°C with 220 rpm agitation.

Twenty 2-liter flasks each containing 120 grams of F204 (solid medium) of the following composition:

Twenty 2-liter flasks each containing 120 grams of millet and 120 milliliters of a stock solution consisting of:

	per liter
Ardamine PH(**)	33.0 g
Sodium tartrate	6.6 g
$FeSO_4 \cdot 7H_2O$	0.66 g
Monosodium glutamate	6.6 ml
Corn oil	6.6 ml

no pH adjustment

**Yeast autolysate available from Yeast Products Inc. Clifton, New Jersey

are autoclaved for 20 minutes at 122°C and then reautoclaved with 80 milliliters of water for another 20 minutes at 122°C. the flasks are allowed to cool, then inoculated with 20 milliliters of seed medium prepared as above described, and the inoculated flasks incubated at 25°C under static conditions for 14 days.

One liter of methanol then is added to nineteen 2-liter flasks of solid fermentation medium and the contents of the flasks are combined, stirred and filtered. The spent cake is then extracted twice with 6 liters of methanol, filtered, and the three filtrates combined and concentrated to 3 liters.

The concentrate is diluted with one liter of water and extracted twice with 3 liters of ethyl acetate. The two 3-liter ethyl acetate extractions are combined, dried with sodium sulfate and concentrated to 100 milliliters.

The 100 milliliter concentrate is adsorbed onto silica gel and the dried silica gel then applied to a 500 ml silica (EM Science, Silica Gel, 60-200 mesh) gel column and eluted with ethyl acetate, followed by ethyl acetate/methanol (9:1). The eluates are tested for presence of antibiotic by determining activity against Candida albicans.

The antibiotic rich eluates are combined, concentrated to an oil and dissolved in 200 milliliters of methylene chloride/hexane/methanol (10:10:1) and combined with 40 ml Sephadex LH-20 which is prepared by soaking overnight in methanol and washing twice with 200 milliliters of 10:10:1

methylene chloride/hexane/methanol. After soaking for a few minutes, the supernatant is removed via filtration and the Sephadex LH-20 washed with 200 milliliters of 10:10:1 methylene chloride/hexane/ methanol and filtered.

The Sephadex LH-20 beads are washed twice with 200 milliliters of methanol, and the methanol washes combined and concentrated.

The combined concentrate is placed on 200 milliliters of silica gel (EM Science, Kieselgel 60, 230-400 mesh) and eluted with ethyl acetate/methanol (75:25) to recover in the eluate biologically active materials. The biologically active material is dried onto silica gel and chromatographed on a silica gel column using ethyl acetate/methanol in a step gradient elution. The biologically active component eluting with 50:50 ethyl acetate/methanol is chromatographed on a DuPont Zorbax ODS column using a solvent mixture of 50:50 acetonitrile/water at a flow rate of 20 ml/minute and monitoring at 210 nm. Fractions are collected and evaluated employing analytical HPLC and antifungal activity. Fractions based on HPLC retention time to Compounds IIA, IIB and IIC.

A 1-gram quantity of Compound IIB is dissolved in 25 ml of dimethyl sulfoxide and the solution added dropwise to a stirred 475 milliliter suspension of P. acidovorans ATCC 53942 cells and the reaction mixture maintained at 37°C for 18 hours whereupon deacylation is found to have been complete with the formation of Compound IB as determined by C. albicans assay.

The reaction mixture is then centrifuged at

6600 G for 20 minutes to separate the cells and to recover Compound IB in the supernatant.

The supernatant is adsorbed on HP-20 resin and Compound IB is recovered from the resin by eluting with methanol and concentrating the eluates. The eluates are combined, diluted with water, charged to a preparation HPLC system equipped with a 50 cm Whatman Partisil 10 SCX Magnum 20 column, and then eluted at 20 ml/min with 0.01 M potassium phosphate (pH=6) buffer and monitored via UV at 210 rm. Cuts rich in the deacylated products are combined and the resulting mixture adsorbed and eluted from HP-20 resin with methanol to remove buffer salts and to obtain purified Compound IB.

EXAMPLE III

In a manner similar to that described in Example II and employing Compounds IIA and IIC obtained as described in Example II, deacylation is carried out to obtain Compounds IA and IC.

Compound I may be acylated to provide new antibiotic substances. Thus, the compounds are useful for preparing semisynthetic derivatives some of which may have particularly desirable properties for utilization as a drug. The preparation and properties of such compounds are the subject of the concurrently filed copending application Serial No. 494,633, (Attorney Docket 18035) previously noted.

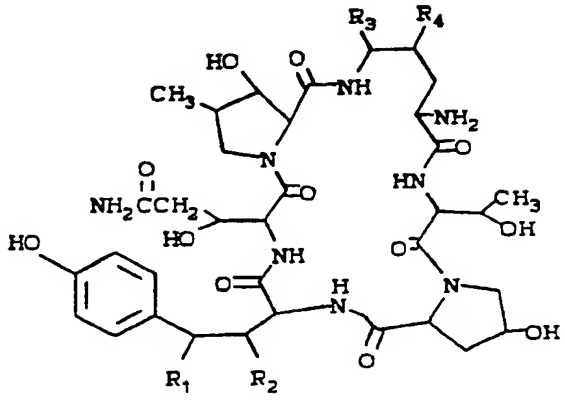
The acylation may be accomplished by reacting Compound I or component thereof with an activated derivative of the acid corresponding to the desired acyl side chain group.

Briefly, Compound I is reacted with an acyl halide, an acid anhydride or an activated ester such as pentafluorophenyl, 3,4,5-trichlorophenyl, p-nitrophenyl or pentachlorophenyl ester of the acid at room temperature in an inert solvent such as dimethylformamide for 15 to 20 hours. At the end of this time, the solvent is vaporized off and the residue purified by a conventional method such as column chromatography on silica gel with ethyl acetate/methanol (3/2) as the eluting agent.

The derivatives, particularly those with acyl groups greater than about 8 carbon atoms are useful to inhibit the growth of pathogenic fungi, both as an antiseptic by controlling growth on surfaces or in treating infections caused by fungi. In particular, the compounds are active against Candida albicans and other fungi causing mycotic infections. Additionally, the compounds may be used for the control of filamentous fungi; especially species infecting plants such as Aspergillus species and Cochliobolus miyabeanus and against fungi infecting paper, paper products, textiles, leather, paint and other consumer goods. The derivatives and their preparation and properties are the subject of the aforementioned concurrently filed copending application (Attorney Docket No. 18035) and the teachings therein are incorporated by reference.

WHAT IS CLAIMED IS:

1. A cyclohexapeptide of the formula



wherein R_1, R_2, R_3 and R_4 independently are hydrogen or hydroxy, and is selected from compounds in which (1) R_2 is hydrogen and R_1, R_3 and R_4 are hydroxy, (2) R_1, R_2 and R_4 are hydrogen and R_3 is hydroxy and (3) R_1, R_2, R_3 and R_4 are hydrogen, and acid addition salts thereof.

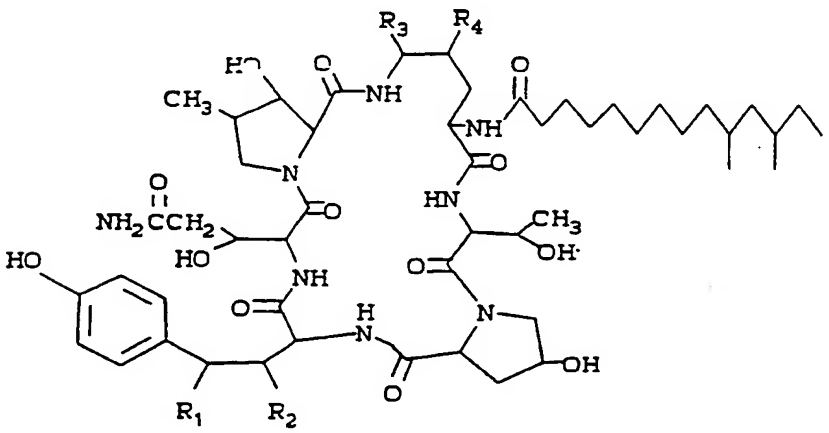
2. A compound according to Claim 1 in which R_2 is hydrogen and R_1, R_3 and R_4 are hydroxy, and is named 1-(4,5-dihydroxyornithine)-4-[4-hydroxy-4-(4-hydroxyphenyl)-2-aminobutanoic acid]-5-(3-hydroxy-glutamine)echinocandin B.

3. A compound according to Claim 1 in which R_1, R_2 and R_4 are hydrogen and R_3 is hydroxy, and is named 1-(5-hydroxy-ornithine)-4-[4-hydroxy-4-(4-hydroxyphenyl)-2-aminobutanoic acid]-5-(3-hydroxy-glutamine)echinocandin B.

4. A compound according to Claim 1 in which R_1, R_2, R_3 and R_4 are hydrogen, and is named 1-(ornithine)-4-[4-(4-hydroxyphenyl)-2-(amino-butanoic acid]-5-(3-hydroxy-glutamine)echinocandin B.

5. A compound according to Claim 1 which is the cyclopeptide in the free base form.

6. A method for preparing a cyclohexapeptide of Claim 1 which comprises subjecting a compound of the formula



in an aqueous medium to a deacylating enzyme produced by a microorganism of the family Pseudomondaceae until substantial deacylation is accomplished wherein in the foregoing formula, R_1, R_2, R_3 and R_4 independently are hydrogen or hydroxy and wherein said compound is selected from compounds in which (1) R_2 is hydrogen and R_1, R_3 and R_4 are hydroxy, (2) R_1, R_2 and R_4 are hydrogen and R_3 is hydroxy and (3) R_1, R_2, R_3 and R_4 are hydrogen.

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